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(54) WATER INSOLUBLE BIOLOGICALLY ACTIVE COMPOUNDS AND PROCESS FOR THEIR MANUFACTURE

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Abstract of the Disclosure:

The present invention relates to water insoluble biologically active compounds in which a copolymer of vinylene glycol is chemically bound to a biologically active substance, a process for their manufacture and their use, preferably in the affinity chromatography.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A process for the preparation of a copolymer of vinylene glycol and a biologically active substance chemically bound thereto in which

(A) a copolymer of the vinylene carbonate is reacted with a biologically active substance and the cyclocarbonate groups still present are subsequently converted into hydroxy groups, or

(B) the cyclocarbonate groups of a copolymer of the vinylene carbonate are converted into hydroxy groups and

(a) the electrophilic groups contained in or introduced into the copolymer are reacted with a biologically active substance, or

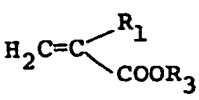
(b) the hydroxy groups are

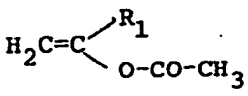
(1) reacted with a compound containing an electrophilic group and then with a biologically active substance, or

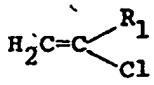
(2) reacted with a biologically active substance carrying electrophilic groups.

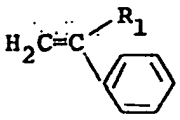
2. A process as claimed in claim 1 in which the copolymer is water-insoluble.

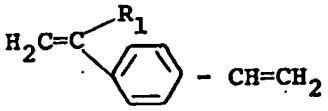
3. A process as claimed in claim 1 in which the copolymer contains at least 55% of monomer units of the vinylene glycol and at most 45% of copolymer units of at least one compound of the general formulae

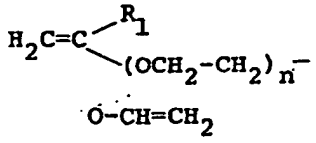


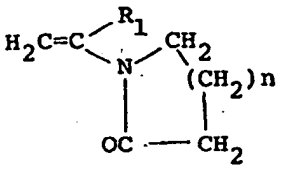


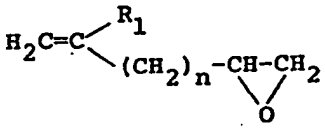


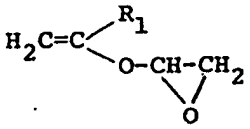


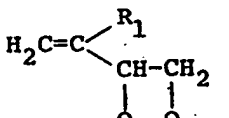












wherein R_1 is hydrogen, methyl, ethyl, R_3 is methyl, ethyl, propyl and n is a whole number between 1 and 4.

4. A compound of a copolymer of vinylene glycol and a biologically active substance chemically bound thereto, whenever obtained according to a process as claimed in claim 1, claim 2 or claim 3 or by an obvious chemical equivalent thereof.

5. A process as claimed in claim 2 in which the biologically active substance is an enzyme, an activator, an inhibitor,

an antigen, an antibody, a plasma protein, a blood group substance, a phythemagglutinine, an antibiotic, a vitamin, a hormone, a peptide, an amino acid or a natural or synthetic effector.

6. A process as claimed in claim 3 in which the comonomer units are present in an amount of 5 to 20%.

7. A process as claimed in claim 1 in which the reaction is carried out according to reaction (A).

8. A compound of the copolymer of vinylene glycol and a biologically active substance chemically bound thereto, whenever obtained according to a process as claimed in claim 4, claim 5 or claim 6 or by an obvious chemical equivalent thereof.

9. A process as claimed in claim 1 in which the reaction is carried out according to reaction (B) (a).

10. A process as claimed in claim 1 in which the reaction is carried out according to reaction (B) (b).

11. A compound of a copolymer of vinylene glycol and a biologically active substance chemically bound thereto, whenever obtained according to a process as claimed in claim 9 or claim 10 or by an obvious chemical equivalent thereof.

The present invention relates to water insoluble biologically active compounds and to a process for their manufacture.

Object of the invention are new compounds of biologically active substances and water-insoluble high-molecular weight compounds, a process for their manufacture and their use preferably in the affinity chromatography.

In the recent years, a new technique within the biochemical working methods increasingly gained in use. Its primary characteristic is the use of the affinity of carrier-bound, biologically active substances for selective reactions.

Through a specific complex formation of the carrier-bound substance with a second substance in a mixture, this substance can be eliminated from the mixture and if desired, subsequently isolated by desorption.

Carrier-bound enzymes have the advantage that they allow the transformation of a substance in continuous processes and the reaction products obtained to be free of enzymes.

In the biochemical, enzymatic analysis, carrier-bound water-insoluble enzymes can repeatedly be used as reactants.

Because the enzymes have affinity not only to the substrates, but also to the specific inhibitors, the affinity chromatography on carrier-bound enzymes proved to be especially favorable for the obtention of enzyme inhibitors. On the other hand, inhibitors bound to water-insoluble matrices permit the preparative obtention of the corresponding enzymes.

As so-called immune adsorbents, antigens or antibodies are bound to water-insoluble matrices whereafter the corresponding antibodies or antigens can be isolated.

Biologically active substances in the sense of the inven-

tion are natural and synthetic substances acting in vivo and in vitro, which belong to the wide range of enzymes, activators, inhibitors, antigens or antibodies, vitamins and hormons. These biologically active substances representing the active principles of the water-insoluble biologically active compounds of the invention are called effectors.

Most of the carrier-bound effectors so far described are essentially more stable than the corresponding biologically active substances in solution.

Suitable carrier materials, so-called matrices, are advantageously substances that are insoluble in aqueous systems and exhibit an unspecific adsorption as low as possible. To this effect, hydrophobic, hydrophilic and ionic interactions between the matrix and the reactant of the effector should as largely as possible be avoided. A bond with substances to the effector, which is undesired, for example of those which are no specific reactants of the effector, should be excluded.

The matrices so far used as carriers for biologically active substances are on the one hand those that bind the effectors by physical adsorption, for example polystyrene, active charcoal and glass beads, and, on the other hand, those that form a covalent bond with the effectors, for example vinyl polymers as homo- and copolymers, for example polyacrylic acids, polyacrylic acid amides and amino-, carboxy- or sulfonyl-substituted polystyrene, cellulose and its derivatives and natural and synthetic polypeptides and proteins. Because of the equilibrated interaction between the matrix and the effector, carbohydrates, especially cellulose, dextrane, starch, agar and their derivatives, are widely used as matrices in aqueous sy-

stems, even though the carboxyl groups frequently contained in these natural substances are considered to be troublesome because of their unspecific affinities. Moreover, these substances have a relatively poor thermal and chemical stability.

Many of these disadvantages could be eliminated by using polyvinylene glycol, a synthetic polymer, as a matrix. Polyvinylene glycol, also called polyhydroxymethylene, is prepared by acid or basic hydrolysis from polyvinylene carbonate. Each carbon atom bears a hydroxyl group and a hydrogen atom. The uninterrupted -C-C-bond gives the carrier an especially high stability.

Now, it was found that instead of polyvinylene glycol vinylene glycol copolymers are advantageously used as carrier matrix. By incorporating suitable comonomer units into polyvinylene carbonate by polymerization, the physical and chemical properties of the polyvinylene glycol prepared therefrom can be varied to a large extent.

So, for example, the hydrophilic property or "swelling" of the polyvinylene glycol can be modified by the incorporation of hydrophobic monomers, for example ethylene, vinyl chloride or styrene into the polyvinylene carbonate by polymerization. By the copolymerization of vinyl acetate, a vinylene glycol group is replaced in the saponified end product by a vinyl alcohol group, which increases the swelling property in an aqueous medium.

Comonomers, for example diethylene glycol divinyl ether and divinyl benzene enable the cross-linking and thus the improvement of the mechanical properties of the carrier. By incorporating electrophilic functions, for example oxirane

groups (epoxi groups) from vinylglycidyl ether comonomers by polymerization, the matrix can directly be reacted with nucleophilic functions ($-NH_2$, -OH etc.). Carboxyl groups can be introduced through acrylic acid ester comonomers. When the copolymerization is completed, the ester groups are saponified to carboxyl groups. The introduction of $-NH_2$ or -COOH groups allows the activation by means of carbodiimides, isoxazolium salts, glutar aldehydes etc. Copolymers of vinylene glycol/vinyl alcohol have a higher specific surface and so an increased binding capacity for, e.g. proteins on the surface of the polymer powder compared to a vinylene glycol homopolymer.

The present invention provides biologically active compounds from a preferably water-insoluble copolymer of the polyvinylene glycol and biologically active substance bound thereto with the maintenance of its biological activity. In these compounds, the carrier matrix is a polyvinylene glycol copolymer containing up to 45 %, preferably 5 - 20 %, of a comonomer. The biologically active substances are enzymes, activators, inhibitors, antigens or antibodies, other plasma proteins, blood group substances, phythemagglutinines, antibiotics, vitamins or hormons, peptides or amino acids or synthetic effectors.

The polymer carrier and the biologically active substance are bound to each other either directly or via a side chain (Spacer) in a covalent bond.

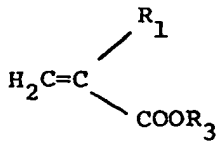
To bind the biologically active compounds via a side chain (Spacer) is advantageous when the molecular weights of their affinity partners are very different, or when in a biospecific process proteins having a very high molecular weight or those comprising several sub-units take part. Spacers are

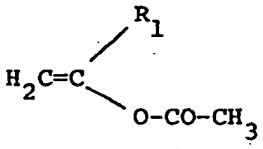
side-chains of a certain lengths anchored to the polymer matrix which can be obtained by polymerization, by addition or incorporation (linkage of bi- or polyfunctional compounds - for example tripeptide - to the matrix) or by incorporation of functional groups and stepwise prolongation.

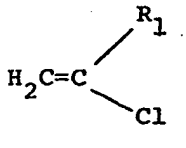
Object of the invention are furthermore processes for the covalent bond of the biologically active substances to the carrier.

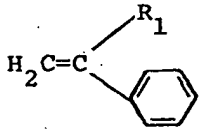
For this purpose, a series of generally known reactions is available.

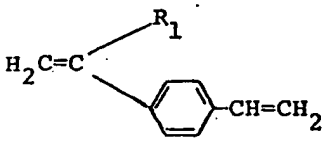
They firstly concern the processes for the manufacture of the polyvinylene glycol copolymer. These processes are characterized in that vinylene carbonate is polymerized with comonomers of the general formulae:

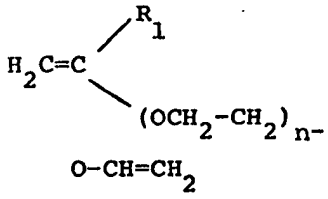


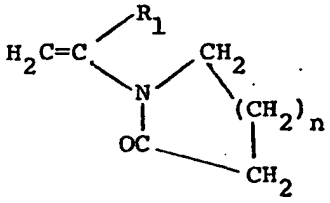


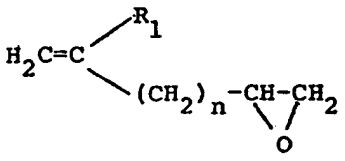


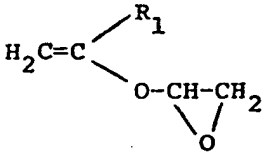


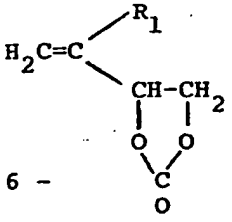












in which R_1 is hydrogen, methyl, ethyl, R_3 is methyl, ethyl, propyl and n is a whole number between 1 and 4.

The polymerization of the polyvinylene carbonate occurs with the use of 45 % of comonomers, preferably 5 - 20 %.

The biologically active compound can be prepared, for example by introducing an electrophilic group either into the biologically active substance or into the polyvinylene glycol copolymer through which the biologically active substance reacts with the polyvinylene glycol copolymers. The introduction of the electrophilic groups into the carrier matrix occurs either by copolymerizing vinylene carbonate with comonomers containing electrophilic groups or by reacting the carrier matrix with low molecular weight compounds carrying activating electrophilic groups.

Generally, the chemical coupling of a reactant to the carrier matrix is simple when the one side carries the nucleophilic functions and the other side the electrophilic functions. Reactive, nucleophilic functions are above all amino, sulfhydryl and hydroxyl groups, which are generally already contained either in the matrix or in the reactant. Electrophilic functions have to be introduced. To this effect, the carboxyl groups can be transformed into acid halides, acid azides, acid anhydrides, imidazolides or directly be activated with carbodiimides. Reactive electrophilic groups are also isocyanate, isothiocyanate, diazonium groups or cyclic imidocarbonate esters. Especially suitable is the introduction of reactive groups by means of copolymerization.

By the introduction of monomers having epoxy groups, for example epoxyalkylvinyl ethers, into the polyvinyl carbonate

by polymerization and because of the very big differences in the saponification speed between cyclic carbonate and epoxy groups, it is easy to obtain a polyvinylene glycol matrix having reactive epoxy groups which can be reacted with nucleophilic functions. So, the direct bond of a biologically active substance and/or the introduction (prolongation) of a side chain (Spacer) is possible.

The further advantage of the use of copolymers is the possibility to introduce directly by polymerization electrophilic functions which allow a reaction according to known methods.

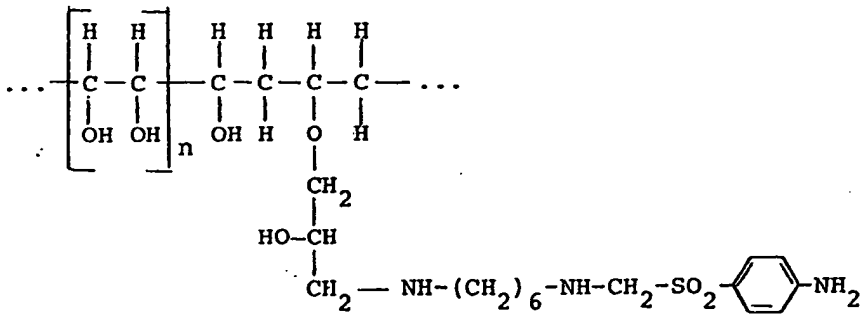
The copolymerization of vinylene carbonate with ethyl acrylate and following saponification of the polymers gives a matrix having -OH- and -COOH-functions. The carboxyl groups can be activated by means of carbodiimides, whereupon an amide linkage to the amino groups of the effector occurs. Carboxyl groups further allow the formation of amide bonds by means of the isoxazolium salts suggested by Woodward.

A further method is the coupling of biologically active compounds to monomers containing carboxyl groups by means of N-ethoxycarbonyl-2-ethoxy 1,2-dihydroquinoline (EEDQ).

The carboxyl groups can be esterified according to known methods, the ester then be converted into hydrazide and the azide resulting therefrom be linked via the amino group of the effector protein to the polyvinylene glycol matrix.

When the comonomer polymerized into the matrix contains amino groups, which can be set free by following reactions, arylamino groups can be introduced by means of vinylsulfon derivatives containing arylamino groups or of sulfuric acid

esters of β-hydroxyethyl sulfones. Then the aryl amino groups can be diazotized according to known methods and bound to reactive groups of an effector, for example



In this way, a prolongation of a Spacer is possible.

Proteins can also be bound via the amino groups by means of glutardialdehyde.

The linkage of the effector to the matrix after an activation of the hydroxyl or amino groups of the polyvinylene-glycol copolymer is easy using cyano halides, preferably bromocyane and effecting a following reaction of the biologically active effectors containing the amino groups through these activated groups.

The effector can also be bound to the polyvinylene glycol or polyvinylene glycol copolymer matrix by acylating the hydroxyl groups with bromoacetyl bromide, followed by the aklylation of the amino group of the effector.

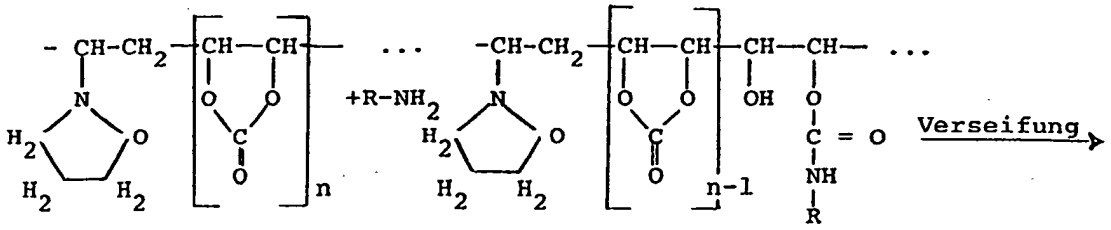
Similar courses of the reaction can be achieved, for example, by reacting the hydroxyl groups of the carrier with reactive triazines, one part of the reactive groups of the triazine reacting with the polyvinyleneglycol compound, another one with the amino groups of the effector.

Diazotizable aromatic amines which can react through a further reactive group with the hydroxyl groups of the carrier permit on the other hand the coupling with capable activated amino acids, for example the tyrosine or histidine radicals of the protein effector.

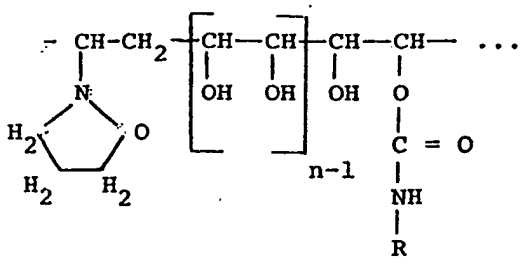
Vinylsulfone derivatives containing arylamino groups and sulfuric acid semi esters of ß-hydroxylethyl sulfones can be reacted with the hydroxyl groups of the carrier. They allow the effector to be bound according to the precited diazotisation reaction.

Especially stable ether bonds are obtained in the reaction of the hydroxyl groups of the polyvinyleneglycol copolymer with epoxides which do not form ions and which contain at least two reactive groups, such as the epihalohydrines or the polyepoxides, for example epichlorohydrine or bisepoxides.

A further mode of modification is the introduction by polymerization of comomomers into the polyvinylene carbonate chain (for example of vinylpyrrolidone) followed by the partial reaction of the cyclocarbonate rings of the polyvinylene carbonate with an amine, for example hexamethylene diamine, to give a polyvinylene carbonate copolymer substituted by a Spacer or an effector through an urethane compound. The residual cyclocarbonate groups are then saponified to hydroxyl groups:



Polyvinylencarbonat



Beside the cited examples of methods for the production of the covalent bond between the carrier matrix and the protein effector or other effectors there are further methods which lead to the reaction of the hydroxyl groups or of given groups of the copolymer with an effector so giving rise to the covalent bond between them, for example the known reaction with complex-forming metal compounds, for example titanium compounds. All these methods are known to those skilled in the art.

When low-molecular weight, biologically active compounds are to be bound, it is advantageous not to activate the carrier but the effector.

Principally, all the methods can be used which are known in the macromolecular chemistry for the modification of synthetic or natural macromolecular compounds.

The polyvinylene glycol copolymers are distinguished beside their chemical and thermal stability by advantageous properties for technical procedures which makes them superior to the carrier matrices on the basis of natural carbohydrates previously deemed to be optimum. For example, they can be prepared in the form of fibers, threads, foils or spheric particles, so that the most suitable form can be choosen depending on the intended application purpose of the effector to be bound thereto. Preferably the copolymers are in the form of finely divided powders having a high specific surface.

A further advantage of the polyvinyleneglycol copolymers over the known carrier materials is the controllability on the industrial scale of the dimension of the surface accessible to the linkage of the effectors or the Spacers.

The biologically active compounds according to the invention can be used for most of the methods which have become known so far for other effectors bound to hydrophilic, water-insoluble carriers. So, enzymes can be made water-insoluble . The insoluble enzymes are increasingly used for the determination of substrates in analysis automatons and as so-called enzyme electrodes. Because of the higher stability, a series of carrier bound enzymes suits for the enzymatic reactions on a technical scale.

Because of their property as specific adsorbents, carrier-bound, biologically active substances have found wide application in the affinity chromatography. Carrier bound, natural or synthetic enzyme inhibitors enable the high-grade purification of enzymes, while the enzymes proved to be excellently suitable effectors especially for the obtention of natural en-

zyme inhibitors from crude extracts. Carrier-bound water-insoluble antigens are used for the isolation of the adherent antibodies which are thus obtained in a state free of other serum constituents and antigens. In the affinity chromatography, antibodies incapable of being precipitated and those which cannot be precipitated because of their low concentration in the serum can be isolated and be determined quantitatively.

The following Examples illustrate the invention:

E X A M P L E S:

Manufacture of the vinylene carbonate copolymers

The vinylene carbonate used in the preparation of the copolymers (CP) was boiled under reflux, before its use, for an hour over sodium boron hydride (100 parts by weight of vinylene carbonate to 2 parts by weight of $NaBH_4$) under a pressure of 33 mm, distilled at 75°/33mm over a 50 cm long silver-sleeve column filled with Raschig glass rings and used as straight as possible for the copolymerization. Also, the comonomers used were purified before by distillation.

E X A M P L E 1:

Copolymer of vinylene glycol/vinyl alcohol from saponified copolymer of vinylene carbonate/vinyl acetate

a) 0.05 Part by weight of azobisisobutyronitrile are dissolved under nitrogen in 9 parts by weight of vinylene carbonate and 1 part by weight of vinyl acetate. The monomer mixture is filled under nitrogen in a flattened screw cap aluminum tube (4 mm thick, 60 mm large, 150 mm long) (total volume of the mixture filled in: about 35 ml), sealed under N_2 and hung into a 50° C hot water bath during 48 hours. Hard, brittle plastic plates are obtained which

are precomminuted in a crushing mill. Then, the granules are heated under a pressure of 1 - 2 mm for 5 hours at 120° C, to separate the part of the monomer which has not been polymerized. The demonomerized granules are further ground in a mill to get a grain size of <0.1 mm, introduced into 500 parts by weight of 0.5 N sodium hydroxide solution, mixed at 20° C during 1 hour by means of a high-speed stirrer, whereupon the carbonate and acetyl groups are saponified. The water-insoluble CP-vinylene /glycol/vinylalcohol is suction-filtered, thoroughly freed from inorganic salts with water and lyophilised.

Yield: 4.7 parts by weight of CP-vinylene glycol/vinyl alcohol having a specific surface measured according to BET of 34 m^2g^{-1}.

b) Process as described under 1a), using instead 7 parts by weight of vinylenecarbonate to 3 parts by weight of vinyl acetate.

Yield: 4.1 parts by weight of CP-vinylene glycol/vinyl alcohol having a specific surface of the lyophilised polymer powder of 62.5 m^2g^{-1}.

c) Comparison test as described under 1a), differing in that 10 parts by weight of vinylene carbonate and no comonomer are used.

Yield: 4.7 parts by weight of homopolymer of vinylene glycol having a specific surface of the lyophilised polymer powder of 20.5 m^2g^{-1}.

E X A M P L E 2:

Copolymer of vinylene glycol/vinylglycidyl ether

A mixture of 7 parts by weight of vinylene carbonate, 3

parts by weight of vinylglycidyl ether and 0.1 part by weight of azobisisobutyronitrile is heated to 50° C for 3 days under N_2 in flattened aluminum tubes as described under 1a). As described under 1a) the plastic plates obtained are comminuted, demonomerized, ground to a grain size of < 0.1 mm, suspended in 500 parts by weight of ice cold 0.5 N NaOH using a high-speed stirrer during 30 minutes, the saponified polymer is immediately centrifuged off, suspended in ice-water, neutralised to pH 7 with 2 N H_2SO_4 while cooling with ice, suction-filtered, washed with ice-water and lyophilised.

Yield: 3.9 parts by weight of CP-vinylene glycol/vinylglycidyl ether containing 1.3 milliequivalent of oxirane groups/g. (determined according to "Praktikum der makromolekularen organischen Chemie", page 221, by Braun, D., Cherdron, H., Kern, W. Hüthig Verlag, Heidelberg 1966).

E X A M P L E 3:

Aminosubstituted copolymer-carrier material

3.9 parts by weight of CP-vinylene glycol/vinylglycidyl ether (of example 2) are suspended in 100 parts by weight of H_2O by means of the Ultra-Turrax stirrer and 10 ml of 30 % ammonia are added. The mixture is stirred at 50° C for again 10 hours by means of a small magnetic stirrer, suction-filtered and thoroughly washed with water and lyophilised.

Yield: 3.5 parts by weight of copolymer-carrier material containing 0.9 m of equivalent amino groups/g in the form of 3-amino-2-hydroxy-propyl groups (obtained by reacting ammonia with glycidyl groups).

E X A M P L E 4:

CP-vinylene glycol/acrylic acid

As described in Example 1a), 9 parts by weight of vinylene carbonate, 1 part by weight of acrylic acid ethyl ester and 0.05 part by weight of azobisisobutyronitrile are polymerized under N_2, demonomerized and ground to a grain size of < 0.1 mm, saponified, suction-filtered, washed and lyophilised.

Yield: 5 parts by weight of hydrophilic polymer powder containing 1.1 milliequivalent of carboxyl groups per gramme of carrier having a specific surface according to BET of 27.2 m^2g^{-1}.

E X A M P L E 5:

Polyvinylene glycol substituted by ω-aminohexyl groups

5 parts by weight of CP-vinylene glycol/acrylic acid (Example 4) are suspended in 100 ml of H_2O by means of the Ultra-Turrax stirrer, cooled to 5° C, 2.5 g of N-cyclohexyl-N'-(N-methylmorpholino)-ethyl-carbodiimide-p-toluene-sulfonate are added, the mixture is stirred for 30 minutes at pH 5.1 and at 5° C, filtered off and rapidly washed with water. The activated carrier is then immediately suspended in a solution of 5 g of hexamethylene diamine in 100 ml of water adjusted to pH 7.5 with 2 N HCl and cooled to 5° C and further stirred under these conditions using a magnetic stirrer, suction-filtered, thoroughly washed with water and lyophilized.

Yield: 4.5 parts by weight of polyvinylene glycol substituted by ω-aminohexyl groups containing 0.7 milliequivalent of aminogroups per gramme of carrier.

E X A M P L E 6:

Reaction of CP-vinylene glycol/ vinyl alcohol of Example 1a) having a specific surface according to BET of 34 m^2g^{-1}

a) with epichlorohydrine:

50 g of a CP-vinylene glycol/vinyl alcohol prepared according to Example 1a) are suspended in 1 liter of 2 N NaOH, 250 ml of epichlorohydrine are added to the suspension and the mixture is stirred at 55 - 60° C for 2 hours. After a short time, the pH of the suspension drops to 10 - 11. By adding NaOH this pH value is maintained for another hour. After a reaction time of 2 hours, the solid substance is suction-filtered, washed with water, acetone and finally again with water.

b) with hexamethylene diamine:

50 g of hexamethylene diamine are dissolved in 1.5 l of water and HCl is added to reach pH 10. The CP-vinylene glycol/vinyl alcohol activated according to 6a is added to the solution and stirred at 50 - 55° C for 6 hours. Then, the product is suction-filtered and washed with water until free of hexamethylene diamine.

c) with 1-aminobenzene-4-ß-hydroxyethylsulfonsulfuric acid ester:

The product obtained according to 6b) is stirred at 55° C and at pH 10 for an hour with 50 g of 1-aminobenzene-4-ß-hydroxyethylsulfonsulfuric acid ester. Then, the solid substance is filtered off, washed with water, acetone and again with water.

d) Diazotization:

10 g of the product obtained according to Example 6c)

are washed with 200 ml of 0.1N HCl on the suction filter and then suspended in 300 ml of 0.5 N HCl. 0.1 N $NaNO_2$-solution is added to the suspension at 0 - 4° C while stirring until a slight nitrite excess is stated by means of potassium iodide starch paper in the diazotation. After 10 minutes, the mixture is suction-filtered and the residue is washed with ice water and then with 0.15 M sodium phosphate buffer at pH 7.5 at 0 - 4° C.

e) Covalent bond with protein:

0.8 g of albumin are dissolved in 350 ml of phosphate buffer at pH 7.5, cooled to 4° C and the product prepared under 6d) is added. The suspension is stirred at 4° C for 20 hours, filtered off and the solid substance is washed with 1 M NaCl and phosphate-buffered sodium chloride solution (PBS) (aqueous 0.9 % NaCl-solution having a content of 1/15 mol of Na_2HPO_4-KH_2PO_4 buffer of pH 7.2).

The filtrate and washing liquors are tested for albumin according to the method of the radial immuno diffusion. 60 mg of albumin are bound to 1 g of the carrier so prepared.

E X A M P L E 7:

Reaction of CP-vinylene glycol/vinyl alcohol of Example 1b having a specific surface according to BET of 62.5 m^2g^{-1}.

According to the reactions described under 6a to e, 80 mg of albumin can be bound to 1 g of activated carrier quantitatively.

E X A M P L E 8:

Reaction of the homopolymer vinylene glycol of example 1c having a specific surface according to BET of 20.5 m^2g^{-1}

According to the reactions described under 6a) to e) 45 mg

of albumin can quantitatively be bound to 1 g of activated carrier.

E X A M P L E 9:

Vinylene glycol/vinylpyrrolidone copolymer substituted by ω -aminohexyl groups

0.05 part by weight of azobisisobutyronitrile are dissolved under nitrogen in 9 parts by weight of vinylene carbonate and 1 part by weight of vinylpyrrolidone and, as described in Example 1a), polymerized and worked up.

After demonomerization, the granules are dissolved to give a 12 % by weight dimethylformamide solution and this soltution is introduced through a nozzle into a methanol precipitation bath under a pressure of 15 atmg. A copolymer of vinylene carbonate and vinylpyrrolidone precipitates in fibrillate form, it is suction-filtered, washed with methanol and resuspended in 200 parts by weight of methanol. 6 parts by weight of hexamethylene diamine, dissolved in 50 parts by weight of methanol are added, the suspension is stirred for 2 days at room temperature, suction-filtered and washed with methanol. The washed filter residue is then suspended in a solution of 10 parts by weight of sodium methylate in 300 parts by weight of 96 % methanol during 4 days at room temperature, washed with methanol and then very thoroughly with water and lyophilised.

Yield: 5,0 parts by weight of a copolymer of vinylene glycol and vinylpyrrolidone substituted by ω-aminohexyl groups through urethane bonds containing 1.6 m of equivalent NH_2-groups/g of carrier.

E X A M P L E 10:

Linkage of IgG (Immunoglobulin a) to the copolymer substituted by ω-aminohexyl groups of Example 9 by means of succinic acid anhydride and water-soluble carbodiimide

5 parts by weight of the ω-aminohexyl-substituted carrier prepared according to Example 9 are succinoylated at 10° C and at pH 6 for 4 hours with 2.5 parts by weight of succinic acid anhydride which are suspended in 200 parts by weight of water. The pH is adjusted with 2 N NaOH. After washing the solid substance with water, the product is stirred with 1.25 parts by weight of N-cyclohexyl-N'-(N-methyl-morpholino)-ethyl)-carbodiimide-p-toluene-sulfonate at pH 5 and 5° C for 30 minutes, filtered off and rapidly washed with ice water.

0.5 part by weight of IgG are dissolved in 150 parts by weight of phosphate buffer of pH 7.5 and stirred with the activated carrier for 24 hours at 4° C. After filtration, the product is washed with 1 M sodium chloride salt and with PBS.

75 mg of IgG are linked to 1 g of carrier in a covalent bond.

E X A M P L E 11:

Copolymer of vinylene glycol/diethylene glycol divinyl ether

0.1 part by weight of azobisisobutyronitrile are dissolved under nitrogen in 9 parts by weight of vinylene carbonate and 1 part by weight of diethylene glycol divinyl ether and heated as described in the Example 1a) in flat aluminum tubes under N_2 to 50° C during 3 days and polymerized. After working-up and saponification as described under 1a), 3.5 parts by weight of the CP-vinylene glycol/diethylene glycol divinyl ether are obtained.

EXAMPLE 12:

Copolymer of vinylene glycol/N-acryloylaminoacetaldehyde-dimethyl acetal

a) 0.05 part by weight of azobisisobutyronitrile are dissolved under nitrogen in 9 parts by weight of vinylene carbonate and 1 part by weight of N-acyloylaminoacetaldehyde-dimethyl-acetal. The monomer mixture is polymerized as described under 1a), worked-up and saponified to give the 4.1 parts by weight of the copolymer of N-acryloyl-aminoacetaldehyde-dimethyl-acetal and vinylene glycol.

10 parts by weight of the copolymer of N-acryloylamino-acetaldehyde-dimethylacetal and vinylene glycol were stirred in 100 parts by weight of 1 N HCl for 4 - 5 hours. The activated carrier was washed with water and phosphate buffer of pH 7.5.

b) 0.5 g of albumin was dissolved in 200 ml of PBS and stirred with the product prepared under a) at 4° C during 14 hours. After filtration the carrier-bound protein was washed with 1 M of sodium chloride salt and with PBS.

1 g of the carrier so prepared binds 40 mg of albumin.